BIOPHYSICAL AND SPECTROSCOPIC STUDIES OF ANTHRAQUINONE DERIVATIVES WITH G-QUADRUPLEX DNAs

A

SYNOPSIS

FOR THE PARTIAL FULFILMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN

CHEMISTRY

SUBMITTED BY:

PRIYA KUMARI

UNDER THE SUPERVISIONS OF

PROF. SURAT KUMAR
Supervisor
Dept. of Chemistry
Faculty of Science

PROF. (Retd.) RITU BARTHWAL
Co-Supervisor
Dept of Biotechnology
IIT, Roorkee

DEPARTMENT OF CHEMISTRY, FACULTY OF SCIENCE
DAYALBAGH EDUCATIONAL INSTITUTE (DEEMED UNIVERSITY)
DAYALBAGH, AGRA-282005
2020

Introduction

In recent years, the problem of cancer is rapidly increasing. It is the main cause of death worldwide. The global cancer occurrence is estimated to have risen to 18.1 million and 9.6 million deaths in 2018 (International Agency for Research on Cancer). There are many ways to treat cancer medicinally. Surgery, radiotherapy, chemotherapy and immunotherapy are the most common treatments to choose. To control this hazardous disease, chemotherapy has been widely used. In chemotherapy, anticancer drugs like Vincristine, Vinblastine, Taxol, etc. are used in clinics (Casaddy & Dourous, 1980). However, chemotherapeutic agents have shown limited antitumor activity due to their non-specific toxicity. Therefore, there has been a growing interest in developing anticancer drug with specific biological activities (Chorawala et al., 2012). It has been reported that the activity of telomerase is suppressed through complexation of G-quadruplex (G4) DNAs with small ligands. Therefore, G–quadruplex are the potential targets for drugs as the over expression of telomerase enzyme is causing cancer (Phan, 2009).

G-Quadruplex

DNA has an important role in genetic information storage and biological processes and there are various topological forms of DNA such as duplex, triplex, quadruplex DNA (Yang & Okamato, 2010). Quadruplex structures are generally formed by guanine-rich sequences found at the 3'-end of telomere and oncogene promoter regions (Jain & Bhattacharya, 2012). Human telomeric DNA consists of $(TTAGGG)_n$ repeats which folds into G-quadruplex structure (Bessi et al., 2012). These quadruplex contain a basic repeating unit that is G-quartet formed by four guanine bases through a cyclic Hoogsteen-hydrogen bonding. These planar G-quartets stack on top of one another forming four-stranded helical strands (Fig 1.1, Rhodes & Lipps, 2015).

Different kinds of loops connect quadruplex strands which are:

- Edgewise loop (Fig 1.2A)
- Diagonal loop (Fig 1.2B)

❖ Propellar loop (Fig 1.2C)

G4-DNA shows polymorphic structure which depends upon the nature of cation such as Na^+ and K^+ (Phan, 2009).

The different G-quadruplex topologies depends upon certain factors such as the *syn/anti* conformation of guanine residues, the relative orientation of G-quartet core, types of loops, sequences and length of telomere DNA and the nature of cation (Xu, 2011). Stability and formation of quadruplex structure depends upon monovalent cations such as K^+ and Na^+. However, K^+ is a better coordinating ion than Na^+ due to lower dehydration energy and higher intracellular concentration (Yang & Okamato, 2010). There exist three possibilities for strand orientation in G-quartet core:

a) All strands in same direction or Parallel stranded structure.
b) Two adjacent strands in one direction and remaining two in opposite direction or Anti-parallel stranded structure.
c) Three strands in one direction and fourth residue in opposite direction or [3+1] Hybrid structure (Phan, 2009).

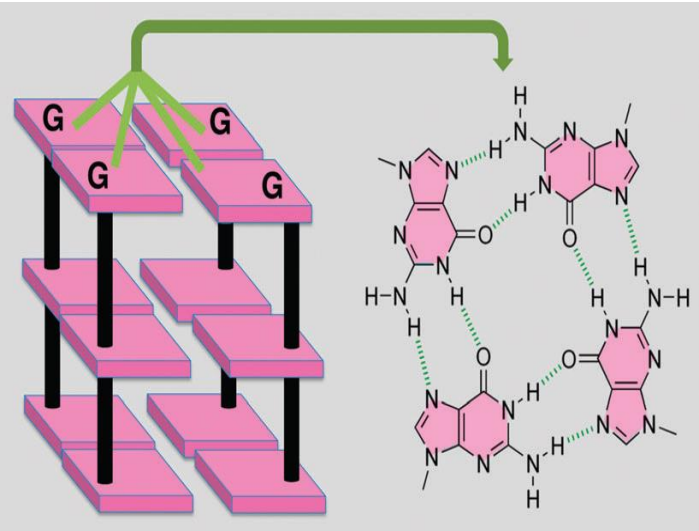
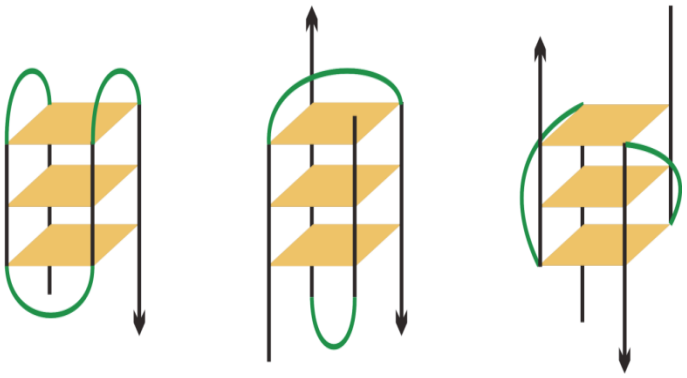


Figure 1.1: Guanine Tetrad Core (Xu, 2010)



A) B) C)

Figure 1.2: Loop Arrangements A) Edgewise B) Diagonal C) Propellar loops (Malgowska et al., 2012)

A parallel G-quadruplex structure has been observed for 11-mer sequence d-(AGGGTTAGGGT) with guanines in anti-conformation in presence of K^+ ion (Parkinson et al., 2002). In Na^+ solution, the G-tetrad structure adopts anti-parallel stranded basket-type structure for 22-mer sequence d-{$AGGG(TTAGGG)_3$} (Wang & Patel, 1993). From NMR studies, a intramolecular hybrid-type mixed parallel/anti-parallel structure has been observed for wild type human telomere 26-mer sequence i.e d-{$TT(TTAGGG)_4$} in K^+ solution in which the one end is covered by T:A:T capping structure (Dai et al., 2007).

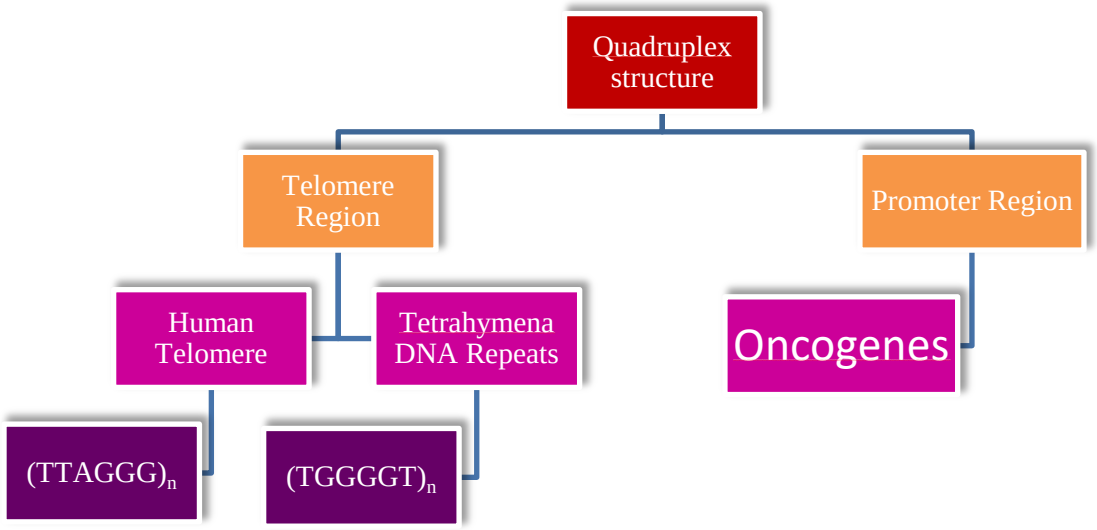


Figure 1.3: Flowchart showing location of Quadruplex structure

Various researchers are working on targeting G-quadruplex DNA with small molecules in order to inhibit the cancer-cell growth based on available two-distinct mechanisms (Fig 1.3). First, by the inhibition of telomerase enzyme activity which catalyzes the elongation of 3'-telomeric end and second, by the inhibition of over-expression of oncogenes like c-Myc, c-Kit involving promoter deactivation (Qin & Hurley, 2008). Hence, G-quadruplex can act as the emerging anti-cancer targets in which the telomerase enzyme inhibition activity is widely studied (Luedtke, 2009). The telomerase activity is inhibited by the formation of G-quadruplex DNA with small ligands (Phan, 2009). Telomerase, a ribo-nucleoprotein is an enzyme which is present in nearly 85 % of cancer cells and regulates their proliferation (Jain & Bhattacharya, 2012).

Anti-Cancer Drugs: Chrysophanol, Rhein and 1,4-bis(3-morpholinopropionamido) anthracene-9,10-dione

Anthraquinone derivatives are widely used in cancer chemotherapy and have been used in a variety of pharmacological activities such as anti-inflammatory, wound healing, analgesic, antimicrobial and antitumor activities (Huang et al., 2008). Anthraquinone (9,10-dioxoanthracenes) contains a planar three-ring aromatic system anthracene that contains two keto groups at 9- and 10-positions and there has been a growing interest towards the development of anthraquinone derivatives with modified activity (Malik & Muller, 2016). Anthraquinone are one of the most promising and attractive compounds because of their ability to target the DNA quadruplex. The first ligand that was shown to stabilize the G-quadruplex and inhibit the telomerase activity was an anthraquinone derivative at an IC_{50}= 23 µM (Tripathi et al., 2016). It has been reported that the apoptosis of cancer cells can be induced by anthraquinone derivatives such as aloe-emodin, emodin, rhein (Lim et al., 2018).

Emodin not only inhibits the viability and invasiveness of ovarian cancer cells but also induces colon cancer cell death. Chrysophanol (1,8-dihydroxy-3-methyl anthracene 9,10-dione) is an anti-inflammatory, anti-viral and anti-tumour anthraquinone but has not been widely studied in cancers (Park et al., 2018). It has been reported that the chrysophanol shows the anti-viral activity against hepatitis B, polio, human

deficiency and hepatitis C (Chang et al., 2013). Rhein (4,5-dihydroxy anthracene-9,10-dione-2-carboxylic acid), an anthraquinone derivative found in the rhizome of rhubarb shows anti-tumour property (Li et al., 2006). It inhibits the proliferation of human breast, lung, colon and glioma cancer cells and also used in the treatment of inflammatory diseases such as osteoarthritis, diabetic nephropathy, etc. (Ye et al., 2013).

Several synthetic di-substituted amido-anthraquinone derivatives also show potent inhibitory telomerase activity and the position of di-substitution regulates their selectivity towards quadruplex DNA over duplex DNA (Huang et al., 2008). Generally, the studies on *in-vitro* cytotoxicity and the telomerase inhibition by 1,4; 2,6; 1,5; 1,8 and 2,7–positions of amido-anthraquinones has been carried out (Perry et al., 1998). The position of side chains on these substituents also influence the mode of binding to DNA for example: 1,4-disubstituted amidoanthraquinones binds through intercalative mode while 2,6-disubstituted amidoanthraquinones binds via threading mode to duplex DNA (Collier & Neidle, 1988).

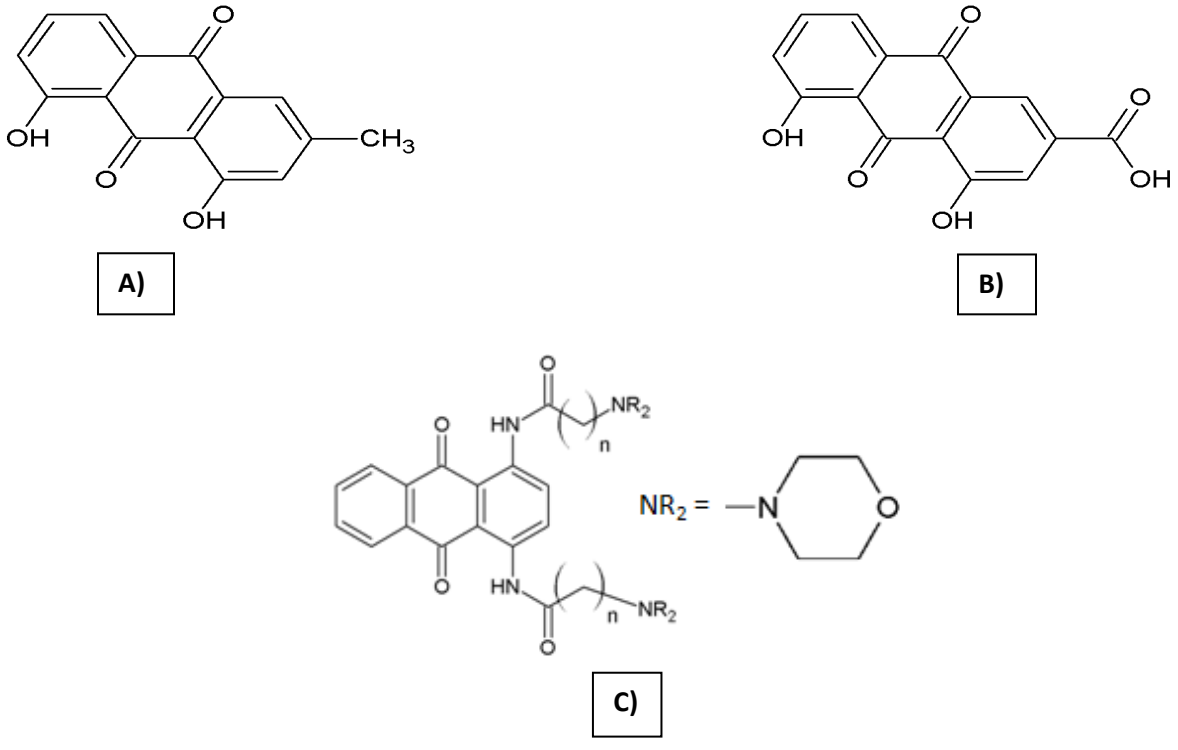


Figure 1.4: Structure of A) Chrysophanol B) Rhein C) 1,4-bis(3-morpholinopropionamido)anthracene-9,10-dione

Role of Nanoparticles

Recent studies have shown that various nanomaterials like silver, gold, silicon dioxide, platinum, zinc, etc. are used to study the anticancer drug–DNA interactions by various methods like spectrofluorometry, electrochemical etc. (Malhotra & Chaube, 2003). Gold nanoparticles are generally used for the immobilization of DNA because of its optical properties in spectrophotometric studies for the detection and estimation of anti-cancer drug activity. Similarly, silver also possesses various properties at its nano-level like catalysis, antimicrobial, bio-molecular diagnosis, drug delivery, antibacterial activity etc. The optical property is unique than others since it is used in the spectrofluorometric studies (Nam et al., 2003). Gold nanomaterials such as gold nanoparticles and gold nanorods (GNRs) act as the promising nanomaterials in the designing of chemical and biochemical sensors for DNA hybridization and cancer cell imaging (Jin et al., 2011). GNRs can adsorb bio-macromolecules that posseses negative charge such as DNA, peptides etc. through electrostatic interactions. Thus GNRs based FRET technique has been developed for monitoring human telomere DNA hybridization and G-Quadruplex formation (Gou et al., 2010).

Analytical Techniques for Detection of Drug-DNA Interactions

Optical Methods

The drug-DNA interactions can be detected by UV-Visible absorption spectroscopy by measuring the changes in the absorption properties of the drug or the DNA molecules. The UV–Vis absorption spectrum of DNA exhibits a maximum at 260 nm. Drug-DNA interactions can be studied by comparing the UV–Vis absorption spectra of the pristine drug and the drug-DNA complexes, which are usually different. The binding with DNA through intercalation results in hypochromism (blue shift) or bathochromism (red shift) (Sirajuddin et al., 2013). As a consequence of intercalative mode of binding that includes a stacking interaction of an aromatic chromophore and the base pair of DNA, the extent of the hypochromism consistent with the strength of intercalative interaction can be observed. (Li et al., 2005)

NMR Spectroscopy

It is based on the fact that atomic nuclei endowed with a property called nuclear spin will align with an applied magnetic field. The degree of this alignment depends not only on the strength of the magnetic field, but also on the type of nucleus and its electronic environment. Every nucleus with spin gives rise to a signal or peak which represents a transition between a ground and an excited state (Kaushik et al., 2016). Each magnetically active nucleus is characterized by different parameters such as chemical shift (the position or frequency of the spectral line), multiplicity (the fine structure observed on the spectral lines), *J*-couplings (the separations within a multiplet), relaxation data and Nuclear Overhauser Effect (NOE) connectivities. All these parameters can be used to deduce detailed structural information about the molecule under study (Dai et al., 2007).

Fluorescence Spectroscopy

It is an emission spectroscopy mostly used to study interactions between ligand molecules and DNA. Highly sensitive technique depends on the experimental conditions and spectral shifts are observed in the emission and excitation spectra of drugs.

Fluorescence Resonance Energy Transfer **(**FRET) is a phenomenon that can be observed when the emission spectrum of the donor molecules (D) is overlapped with the excitation spectrum of the acceptor molecules (A). Under adequate experimental conditions (concentration and distance), the fluorescence observed using the excitation wavelength of the donor corresponds to the acceptor because the emission energy of the donor is transferred to the acceptor (Blouin et al., 2009). In the case of fluorescent intercalating agents, the UV energy absorbed by DNA base pairs can be efficiently transferred to the intercalated fluorescent drug. In the case of the groove interacting agents, FRET is not observed because of the greater distance and also due to the fact that orientation of dipole is not adequate for the energy transfer (Sirajuddin et al., 2013).

Circular Dichroism (CD) Spectroscopy

Circular Dichroism (CD) spectroscopy is a form of light absorption spectroscopy that measures the difference in absorbance of left and right handed circularly polarized light. It is a powerful spectroscopic technique to detect conformational changes and secondary structures of nucleic acids or proteins and to study their interaction with ligands. It monitors the changes in global conformations of DNA structure which are generally induced by modifications in the environment, such as pH, temperature, the nature and/or concentration of counter-ions (Kaushik et al., 2016). The inter-conversion in quadruplex topologies from parallel to anti-parallel structures can easily be studied by monitoring CD spectroscopy. The G-quadruplex conformations (parallel, anti-parallel or mixed) adopted by various short and long guanine-rich sequences can be determined from the position and magnitude of the CD peaks. CD spectra of parallel G-quadruplex is characterized by a positive CD peak at 260 nm and a negative band at 240 nm, whereas a positive peak at around 295 nm and a negative band at 260 nm are indicative of an anti-parallel G-quadruplex conformation (Karsisiotis et al., 2011).

Surface Plasmon Resonance (SPR)

Surface plasmon resonance (SPR) technology, enables the monitoring of a variety of bio-specific interactions of DNA-binding drugs with target DNA in real-time. It has recently become an established method of measuring a variety of bio-molecular interactions in real time. It allows detection of analytes interacting with the ligands immobilized on the sensor chip. It can thus be used to study the interactions of any biological system from oligonucleotide, oligosaccharides, proteins, and lipids to small molecules, viral particles and bacterial cells. It further allows the direct observation of association and dissociation rate constants that contribute to the overall binding affinity (Lin et al., 2005).

Biological Assays

Methyl Thiazolyl Tetrazolium (MTT) Assay

MTT assay is used to measure the viability of the cells. It is a colorimetric assay that utilizes a yellow tetrazolium dye. This dye is converted into a deeply colored product in live cells and is easy to be

quantified. In this method, the yellow colored MTT(3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) is converted to purple colored formazan product. By dissolving in a solvent such as DMSO or isopropanol, formazan gives a "blue" colored product. The absorbance of this colored solution is quantified and analyzed by using a spectrophotometer at wavelengths generally between 500 nm and 600 nm. The conversion of tetrazolium dye to the formazan product occurs in the presence of mitochondrial dehydrogenase enzyme. The mitochondrial dehydrogenase is produced only in active and viable cells. Thus the conversion can be directly related to the number of viable cells and inversely related to the cytotoxicity of the compounds tested (Lee et al., 2011).

Telomerase Repeat Amplification Protocol (TRAP) Assay

The telomerase activity has been associated with cancerous cells and this activity is absent in normal somatic cells. The components of telomerase i.e. human telomerase reverse transcriptase (hTERT) and RNA component hTR inhibits the telomere shortening by adding telomeric repeats in tumour cells and hence proliferating the cells (Huang et al., 2005). The TRAP (telomerase repeat amplification protocol) assay is a popular method to determine telomerase activity in mammalian cells and tissue samples. The TRAP assay includes three steps: extension, amplification, and detection of telomerase products. In the extension step, telomeric repeats are added to the telomerase substrate (which is actually a non-telomeric oligonucleotide) by telomerase. In the amplification step, the extension products are amplified by the polymerase chain reaction (PCR) using specific primers (upstream primer and downstream primer) and in the detection step, the presence or absence of telomerase is analyzed by electrophoresis (Mender & Shay, 2015).

Present State of Knowledge

Raje et al. (2019) examined the interaction of epirubicin and adriamycin (anthracyclines) with G4-DNA [d-{AGGG(TTAGGG)$_3$}] by Fluorescence, Absorption, Circular Dichroism, Surface Plasmon Resonance and thermal denaturation studies. The binding constant (K_b) value was found to be 2.5×10^5 M^{-1} and 5.2×10^5 M^{-1} for epirubicin and adriamycin respectively in their monomeric form. The thermal studies

have shown the thermal stabilization of G4-DNA upto 13 ^{o}C and 11.6 ^{o}C upon binding in the presence of K^+ ions. However, thermal stabilization was not observed in the presence of Na^+ ions. The external mode of binding in grooves and loops of G4 DNA has been indicated by molecular docking experiments (Raje et al., 2019).

Pradeep et al. (2016) investigated the binding site of anti-cancer drug mitoxantrone to the tetra-molecular parallel stranded G-quadruplex structure d-$(TTGGGGT)_4$ by using solution state one and two dimensional NMR spectroscopy. It was found that mitoxantrone (MTX) binds in the two opposite grooves of parallel quadruplex and forms a head-to-tail dimer. This head-to-tail dimer formation was confirmed by observing the specific inter-molecular short distance contacts between the protons of two mitoxantrone molecules of dimer. When mitoxantrone binds, the C4 symmetry of G-quadruplex was sustained, as confirmed by the presence of only four guanine NH peaks corresponding to the four G-quartets. A well-defined complex was formed between mitoxantrone (MTX) and G-quadruplex DNA which was indicated by the 38 Nuclear Overhauser enhancement (NOE) cross-peaks. From the job plot and thermal melting analysis, the binding stoichiometry for mitoxantrone:DNA complex was found to be 4:1 (Pradeep et al., 2016).

Airoldi et al. (2014) studied the interaction of doxorubicin with various polynucleotides [poly(dG-dC)·poly(dG-dC) (polyGC), poly(dA-dT)·poly(dA-dT) (poly-AT) and CT DNA]. The buffer used in this study was phosphate buffer and different spectroscopic techniques such as UV-vis, Circular dichroism and fluorescence spectroscopy have been performed. The results indicated that interaction between DNA and doxorubicin is intercalative mode. It was also found that interaction with AT rich regions involves the transfer of excitation energy to doxorubicin and doxorubicin binds with the same affinity to both AT and GC rich sites (Airoldi et al., 2014).

Lad et al. (2014) constructed a gold based optical nanobiosensor for the determination of toxicological behaviour of two anticancer drugs i.e. mitoxantrone (MTX) and carboplatin. The interactions of drug with DNA were studied by Fluorescence Resonance Energy Transfer (FRET) phenomenon which works as the basic principle for the determination of the drug-DNA interactions by spectrofluorometry. As the drug interacted with DNA, an increase in fluorescence intensity was observed in case of MTX while decrease

in fluorescence intensity in carboplatin case was observed. The paracetamol was used as a control drug in both studies which showed a weak interaction with DNA. The developed method provided a new perspective to the FRET and is expected to be used for monitoring the drug-DNA interactions in near future (Lad et al., 2014).

Kasthuri et al. (2011) attempted to evaluate the binding efficiency of Gold nanoparticle (AuNPs) of 56 nm size with Calf thymus DNA (CT-DNA) through UV absorption studies and fluorescent quenching mechanism of ethidium bromide (EB). It was observed that DNA emitted intense fluorescence in the presence of EB, due to its strong intercalation. A decrease in fluorescence was evidenced when AuNPs were added to the EB intercalated CT-DNA. This is due to the increasing concentration of AuNPs, which in turn would compete with the hydrophobic intercalator, EtBr on the DNA binding sites. Thus it is concluded that the fluorescent quenching of EB by AuNPs showed the interaction between the gold nanoparticle and DNA. The AuNPs-CT-DNA interaction would be highly useful in the quantitative detection of DNA samples even at nano level (Kasthuri et al., 2011).

Hudson et al. (2009) investigated the interaction of actinomycin D with human telomeric G-quadruplex consisting of d-{AGGG(TTAGGG)$_3$}. It has been shown in this study that the actinomycin D could bind and induced changes both in the structure and the stability of Na^+ and K^+ forms of quadruplex DNA. The binding has been correlated with intrinsic association constant which was found to be approximately $2\times10^5\ M^{-1}$ for actinomycin D – G-quadruplex complex and the binding enthalpy of approximately -7 Kcal mol^{-1}. Both Na^+ and K^+ forms of quadruplex structure were stablised by the melting temperature of 79^{o}C when bound with actinomycin D. The actinomycin D has induced changes in both Na^+ and K^+ structural forms. The CD spectroscopy revealed that the phenoxazone ring of actinomycin D showed stacking interaction with G-quartet rather than intercalation between adjacent G-quartet (Hudson et al., 2009).

Nagesh et al. (2009) studied the effect of ionic strength on quadruplex DNA formed by the promoter regions of C-myc and Bcl2 oncogenes and porphyrin drugs were used for studying the site and nature of interactions with quadruplex DNA. Three different porphyrin drugs i.e. TMPyP2, TMPyP3 and TMPyP4 were used and allowed to interact with quadruplex DNA of promoter regions. The effect of different

potassium ionic strengths was studied using fluorescence spectroscopy. It was found that the fluorescence hypochromicity decreased with an increase in the ionic strength in the case of TMPyP4, TMPyP3 and TMPyP2. By fluorescence titration and job plots studies, it was found that four molecules of TMPyP4, two molecules of TMPyP3 and TMPyP2 were interacting with one molecule of quadruplex DNA (Nagesh et al., 2009).

Arora et al. (2008) examined the binding of berberine to human telomeric sequence d-{AGGG(TTAGGG)$_3$} through spectroscopic, calorimetric and molecular modeling experiments. It was established through UV-absorption, fluorescence spectroscopy and isothermal titration calorimetry that the binding affinity of berberine for human telomeric quadruplex was 10^6 M^{-1}. The thermodynamic parameters were also calculated which showed a small negative enthalpy (ΔH) change of -1.7 Kcal mol^{-1}, entropy value with $T\Delta S$ of +6.5 Kcal mol^{-1}, Gibbs free energy (ΔG) change of -8.2 Kcal mol^{-1} and heat capacity (ΔCp) of -94 cal mol^{-1}K^{-1}. The molecular modeling studies showed that berberine bonded to the quadruplex by stacking interactions in the external G-quartet core and the negative charge density on O6 of guanines was stabilized by the positively charged N7 of berberine molecule (Arora et al., 2008).

Qiao et al. (2008) reported an effective approach i.e. FRET (Fluorescence Resonance Energy Transfer) for identifying G-quadruplex binding ligands. In this method, DNA functionalized gold nanoparticles were used as the fluorescence quencher and a carboxyfluoroscein tagged human telomeric sequence (F-GDNA) as the recognition probe. In this a thiolated complementary strand of human telomeric DNA was used which first gets attached to the surface of AuNPs. Then hybridizes with F-GDNA which results in the fluorescence quenching of F-GDNA by the AuNPs. However, upon binding of the quadruplex ligands, the single stranded F-GDNA converts into the G-quadruplex structure and the fluorescence intensity was restored leading to the release of F-GDNA from the surface of AuNPs. Thus the ligands selected by FRET method could induce GDNA to form quadruplex and hence quadruplex-binding ligands can be identified (Qiao et al., 2008).

Qaio et al. (2007) investigated the interactions of DNA with anthraquinone derivatives such as chrysophanol, physcion, 1,8-dihydroxy anthraquinone using Ethidium Bromide (EB) as the fluorescent

probe. Fluorescence quenching and UV-Visible spectroscopy technique was used to study binding constants. The binding constants were evaluated in the presence of EB. The effect of ionic strength was also monitored. It was found out that the three derivatives of anthraquinone drugs bind with DNA by groove binding. For ionic strength measurements, NaCl was used in the concentration range of 0 to 0.3 mol/l and a decrement in the fluorescent intensity and an increment in the binding constant values was observed for DNA-EB-anthraquinone systems. The binding constants of chrysophanol, physcion and 1,8-dihydroxy anthraquinone with DNA–EB complex were found to be 1.64×10^4, 3.04×10^4 and 2.88×10^5 mol^{-1} respectively (Qiao et al., 2007).

Dai et al. (2007) reported the structure of human telomeric G- quadruplex in K^+ solution with the help of NMR studies including NOESY, TOCSY and DQF-COSY. It has been observed that the wild type 26-mer human telomeric sequence d-{TT(TTAGGG)$_4$} exhibit intramolecular structure in K^+ solution which consists of hybrid-type mixed parallel/anti-parallel stranded structures (hybrid-2). The hybrid-2 structure differs from hybrid-1 structure in loop arrangements, strand orientations and capping structures. In hybrid-2 structure, TAT triple capping structure is present which covering the one end of this structure and reported to be effective in selective stabilization (Dai et al., 2007).

Perry et al. (1998) studied the inhibitory effect of 1,4 and 2,6-disubstituted amidoanthracene-9,10-dione on human telomerase and Taq polymerase enzyme and examined their *in-vitro* toxicity on several tumour cell lines. It was found that 2,6-disubstituted compounds are less cytotoxic than 1,4-disubstituted compounds. Among all the compounds those having –$(CH_2)_2$- side chains which are terminating into basic groups such as piperidine exhibited inhibitory activity on telomerase at $^{tel}IC_{50}$ levels of 4-11 µM. It was also observed that doxorubicin and mitoxantrone showed total inhibition in activity of Taq polymerase enzyme at 50 µM (Perry et al., 1998).

Formulation of Problem

In recent years, the problem of cancer is rapidly increasing. It is the main cause of death worldwide. To control this lethal disease, chemotherapy has been widely used. In chemotherapy, anticancer drugs like Vincristine, Vinblastine, Taxol, etc. have been used in clinics. In general, conventional therapies that are

used cause serious side effects and generally extend the patient's lifespan by a few years only and rate of recurrence is also high. Chemotherapeutic agents have shown limited antitumor activity. So it is required to develop a method to evaluate the biological behaviour of an anticancer drug and there has also been a growing interest to identify the DNA binding behaviour of an anticancer drug.

It has been reported that the activity of telomerase is suppressed through complexation of G-quadruplex (G4) DNAs with small ligands. The components of telomerase i.e. human telomerase reverse transcriptase (hTERT) and a RNA component hTR counteracts telomere (present at the ends of chromosomes) shortening by adding telomeric repeats to the end of chromosomes in ~90% of primary human tumors. This results in continuous proliferation of cells which is a hallmark of cancer. Therefore, G–quadruplex are the potential targets for drug as the over expression of telomerase enzyme is causing cancer. Since the interaction of Chrysophanol and Rhein with duplex DNA has been studied by several techniques but their interaction with quadruplex DNA has not been studied yet in detail. The study of a synthetic 1,4-diamido derivative of anthraquinone will also be carried out. Hence, in this study the interaction of quadruplex DNA with these drugs will be examined by different technique like Fluorescence Resonance Energy Transfer, UV-Visible Spectroscopy, Circular Dichroism (CD) Spectroscopy, Surface Plasmon Resonance (SPR), Nuclear Magnetic Resonance (NMR). Also the gold based nanoparticles can play an important role in determining the binding behavior of these anti-cancer drugs with DNA. Thus, this study will also focus on examining the binding behavior of these drugs with DNA in the presence and absence of gold nanoparticles using UV-Visible Spectroscopy and Fluorescence techniques.

Objectives

a) To prepare and characterize the formation of Quadruplex DNA
b) To evaluate the binding of Chrysophanol, Rhein and 1,4-bis(3-morpholinopropionamido)anthracene-9,10-dione with:

i) Wild Type Human Telomeric DNA (26-mer sequence)

- wtHTel-26: [d-{TT($TTAGGG)_4$}]

ii) Human Telomeric DNA (22-mer sequence)

- HTel-22: [d-{$AGGG(TTAGGG)_3$}]

c) To evaluate the molecular docking study of Chrysophanol, Rhein and 1,4-bis(3-morpholinopropionamido)anthracene-9,10-dione with:

i) Wild Type Human Telomeric DNA (26-mer sequence) (in K^+)

- wtHTel-26: [d-{$TT(TTAGGG)_4$}]

ii) Human Telomeric DNA (22-mer sequence) (in Na^+)

- HTel-22: [d-{$AGGG(TTAGGG)_3$}]

d) To assay the biological activity of Chrysophanol, Rhein and 1,4-bis(3-morpholinopropionamido)anthracene-9,10-dione with cell-lines.

Materials and Methods

Sample Preparation

Drugs Chrysophanol and Rhein will be purchased from H.V. Technologies and the 1,4-bis(3-morpholinopropionamido)anthracene-9,10-dione will be synthesized in the lab. Their respective solutions will be prepared in desired solvent. The DNA solution will be prepared in the phosphate buffer at physiological pH of 7.4. The concentration of DNA would be determined spectrophotometrically using the molar extinction coefficient value.

The proposed DNA sequence for our studies is:

- Wild Type Human Telomeric DNA (26-mer sequence)

- wtHTel-26: [d-{TT(TTAGGG)$_4$}]

- Human Telomeric DNA (22-mer sequence)

  - HTel-22: [d-{AGGG(TTAGGG)$_3$}]

The proposed anticancer drugs for studies are anthraquinone derivatives:

a) Chrysophanol

b) Rhein

c) 1,4- bis(3-morpholinopropionamido)anthracene-9,10-dione

The stock solution of oligonucleotide 26-mer sequence; [d-{TT(TTAGGG)$_4$}] and 22-mer sequence; [d-{AGGG(TTAGGG)$_3$}] will be prepared by dissolving in respective buffer and heated at 95^0C for 10 min. Then allowed to cool for 48 hr at room temperature for proper formation of G-quadruplex structure. The concentration of prepared DNA will be determined spectrophotometrically with the help of molar extinction coefficient (Tariq and Barthwal, 2018).

Methods

Techniques to Study Drug-DNA Interactions

Spectrophotometric Techniques

These titration techniques are useful in the drug-DNA binding studies. In these measurements, a fixed concentration of drug solution will be titrated by the addition of aliquots of DNA solution in increasing concentration. All Spectrophotometric measurements will be recorded using a spectrophotometer (UV or Fluorescence). Binding constant would be calculated by double reciprocal plot which is 1/[Nucleic Acids] vs. 1/[difference in absorbance or emission] and this gives the straight line with an intercept and slope.

$$1/[DNA] = 1/K + 1/[Difference\ of\ absorption\ or\ emission]$$

Circular Dichroism Studies

It is useful in determination of conformational changes in the structure of DNA upon drug binding. It can be conveniently used to monitor the quadruplex formation. DNA shows characteristic positive and negative peaks in CD spectra by which the structural changes can be easily measured. For example, anti-parallel G-quadruplex shows positive and negative peaks around 295 and 265 nm, while parallel G-quadruplex structures shows positive and negative peaks around 260 and 240 nm respectively.

Surface Plasmon Resonance Studies

By using this technology, a wide variety of bio-molecular interactions can be measured in real-time. It allows the detection of analytes interacting with ligands which are immobilized on the sensor chip surface. With the help of this method, the association and dissociation rate constants can be easily observed which contribute to overall binding affinity.

Molecular Modeling Studies

After obtaining the detailed structural details of the drug-DNA complex, molecular modeling studies will be carried out by using AUTODOCK software in order to furnish a refined structure of the drug-DNA complex.

NMR Studies

After obtaining the DNA binding profiles of these drugs, the elaborated structure of at least one of these complexes would be studied by NMR. 2D ^{1}H-NMR including NOESY experiments may be conducted on the proposed drug-DNA complex as reported (Dai et al., 2007).

Cell-proliferation Assay

For cell-proliferation assay, the cells will be cultured in 96-well microplates and allowed to incubate for 72 h. Then, the medium containing various concentrations of drugs will be added and incubated for another 72 h, followed by MTT and TRAP assay, etc.

Synthesis of Gold Nanoparticles

The colloidal forms of gold nanoparticles will be prepared by the citrate reduction of HAuCl4. A 25 ml aqueous solution containing 1.0×10^{-3} mol L^{-1} HAuCl4 will be brought to vigorously boil with constant stirring in a round bottom flask. A 2.5 mL of 38.8×10^{-3} mol L^{-1} trisodium citrate solution will be added quickly to the solution till solution becomes colorless. The resulting solution will be heated for approximately 15 minutes until the color of the solution changes to deep red. Consequently, the solution will be cooled to room temperature under the constant stir. Finally, a deep red monodispersed "pristine" AuNPs obtained which will be used as stock solution of gold nanoparticles (Kumar et al., 2007). After synthesis, these nanoparticles will be characterized using various techniques.

Reaction

$$\mathbf{2AuCl^{4-} + Ctr^{3-} + H_2O \longrightarrow 2Au + 3HCHO + 3CO_2 + 8Cl^- + 3H^+}$$

(Tyagi et al., 2016)

Techniques used for Characterization of Gold Nanoparticles

1. UV-Visible spectroscopy

2. SEM (Scanning Electron Microscopy)

3. XRD (X-Ray Diffractometer)

UV-Visible Spectroscopy

UV-Visible Spectroscopy using Agilent Cary-60 UV-Vis is one of the most important techniques to characterize gold nanoparticles. The absorption behaviour arises due to the surface plasmon resonance, which originates from coherent oscillation of the electrons in the conduction band of the nanoparticles induced by electromagnetic field peaks of pristine gold nanoparticles. SPR of gold nanoparticles results in a strong absorbance band in the visible region (500 nm-600 nm), which can be measured by UV-Vis spectroscopy (Unser et al., 2015).

Scanning Electron Microscopy (SEM)

Further characterization of gold nanoparticles will be done by using Scanning Electron Microscope (Table Tops SEM-3200M). It is a type of electron microscope that images a sample by scanning it with a beam of electrons in a faster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition, and electrical conductivity (Owen & William, 2010).

X-Ray Diffractometer (XRD)

X-ray diffraction is a method used for determining the atomic and molecular structure of a crystal, in which the crystalline structure causes a beam of X-rays to diffract due to many specific planes and directions present in a crystal lattice. It is a useful tool to examine the structure of nanomaterials (materials with structural features of at least one dimension in the range of 1-100 nm). It provides the wavelength of X- ray on atomic scale. It is non-contact and non-destructive technique. X-ray diffractograms of nanomaterials provide a wealth of information from phase composition to crystallite size from lattice strain to crystallographic orientation. “The XRD pattern of a pure substance is, therefore, like a fingerprint of the substance” (Sharma et al., 2012). It is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed towards the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy **Bragg's Law**. This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample (Dutrow & Clark).

$$2d\sin\theta = n\lambda$$

Where, d = Spacing between atomic planes

θ = Angle of diffraction, λ = Wavelength of the reflected X-ray

Future Scope of Research

This study may have implications on structure-based designing of anthracycline drugs as potent telomerase inhibitors. It may be useful in providing information regarding polymorphisms of G-quadruplex sequences and topology of ligand binding. Since, many G-quadruplexes sequences are involved in cell-proliferation and therefore valuable targets for discovery and development of anticancer drugs.

References

- Airoldi M, Barone G, Gennaro G, Giuliani AM and Giustini M, **2014**. Interaction of doxorubicin with polynucleotides: A spectroscopic study. *Biochemistry*. **53**:2197-2207.
- Arora A, Balasubramanian C, Kumar N, Agrawal S, Ojha RP and Maiti S, **2008**. Binding of berberine to human Telomeric quadruplex- spectroscopic, calorimetric and molecular modeling studies. FEBS Journal. **275**:3971-3983.
- Aslanoglu M and Oge N, **2005**. Voltammetric, UV absorption and viscometric studies of the interaction of norepinephrine with DNA. *Journal of Chemical Education*. **29**:477–485.
- Bessi I, Bazzicalupi C, Richter C, Jonker HRA, Saxena K, Sissi C, Chioccioli M, Bianco S, Bilia A, Schwalbe H and Grateri P, **2012**. Spectroscopic, Molecular Modelling, and NMR-Spectroscopic Investigation of the Binding Mode of the Natural Alkaloids Berberine and Sanguinarine to Human Telomeric G-Quadruplex DNA. *ACS Chemical Biology*. **7**:1109-1119.
- Blouin S, Craggs TD, Lafontaine DA, Penedo JC, **2009**. Functional Studies of DNA-Protein Interactions using FRET Techniques. *Methods in Molecular Biology*. **543**:475-502.
- Cassady JM and Douros JD, **1980**. Anticancer agents based on natural product models. *New York: Academic Press*.
- Chang SJ, Huang SH, Lin YJ, Tsou YY and Lin CW, **2013**. Antiviral activity of *Rheum palmatum* methanol extract and chrysophanol against Japanese encephalitis virus. *Archives of Pharmacal Research.*

- Chorawala MR, Oza PM and Shah GB, **2012**. Mechanisms of Anticancer Drugs Resistance: An Overview. *International journal of Pharmceutical Scences and Drug Research*. **1**:1-9.
- Collier DA & Neidle S, **1988**. Synthesis, Molecular Modelling, DNA binding and Antitumor properties of some substituted amido-anthraquinones. *Journal of Medicinal Chemistry*. **31**:847-857.
- Dai J, Carver M, Punchihewa C, Jones RA and Yang D, **2007**. Structure of the Hybrid-2 type intramolecular human telomeric G-quadruplex in K+ solution: insights into structure polymorphism of the human telomeric sequence. *Nucleic Acid Research*. **35**:4927-4940.
- Dutrow BL and Clark CM. An article on X-ray Powder Diffraction (XRD), *Geochemical Instrumentation and Analysis*, Louisiana State University & Eastern Michigan University.
- Gou XC, Liu J and Zhang HL, **2010**. Monitoring human telomere DNA hybridization and G-quadruplex formation using gold nanorods. *Analytica Chimica Acta*. **668**:208–21.
- Huang HS, Chou CL, Guo CL, Yuan CL, Lu YC, Shieh FY and Lin JJ, **2005**. Human telomerase inhibition and cytotoxicity of regioisomeric disubstituted amido-anthraquinone and amino-anthraquinones. *Bioorganic and Medicinal Chemistry*. **13**:1435-1444.
- Huang HS, Huang KF, Li CL, Huang YY, Chiang YH, Huang FC and Lin JJ, **2008**. Synthesis, human telomerase inhibition and anti-proliferative studies of a series of 2,7-bis-substituted amido-anthraquinone derivatives. *Bioorganic and Medicinal Chemistry*. **16**:6976-6986.
- Hudson JS, Brooks SC and Graves DE, **2009**. Interactions of Actinomycin D with human telomeric G-quadruplex DNA. *Biochemistry*. **48**:4440-4447.
- Jain A and Bhattacharya S, **2012**. Recent Developments in the Chemistry and Biology of G-Quadruplexes with reference to DNA Groove Binders. *Current Pharmaceutical Design*. **18**:1917-1933.
- Jin Y, Chen G and Wang Y, **2011**. Gold-Nanorod based FRET assay for selection of G-Quadruplex Binding ligands. *Gold Bull*. **44**:163-169.

- Kumar S, Gandhi KS and Kumar R, **2007**. Modelling of Formation of Gold Nanoparticles by Citrate Method. *Industrial and Engineering Chemistry Research*. **46**:3128-3136.
- Kalanur SS, Katrahalli U and Seetharamappa J, **2009**. Electrochemical studies and spectroscopic investigations on the interaction of an anticancer drug with DNA and their analytical applications. *Journal of Electroanalytical Chemistry*. **636**:93–100.
- Karsisiotis AI, Hessari NM, Novellino E, Spada GP, Randazzo A and Silva MW, **2011**. Topological Characterization of Nucleic Acid G-Quadruplexes by UV Absorption and Circular Dichroism. *Angewandte Chemie*. **123**:10833-10836.
- Kasthuri J, Poornima S and Dinesh JP, **2011**. Evaluation of the Interaction of Gold Nanoparticles with CT- DNA using Spectroscopic and Electrophoretic Techniques. *Journal of Biosciences Research.* **2**:1-4.
- Kaushik M, Kaushik S and Kukreti S, **2016**. Exploring the Characterization tools of Guanine-Quadruplexes. *Frontiers in Bioscience*. **21**:468-478.
- Lad AKN and Agarwal YK, **2014**. SiO2-Based Nanobiosensor Monitoring Toxicological Behavior of Mitroxanthrone in vitro. *Applied Nanoscience.* **4**:523-529.
- Lee MS, Cha EY, Sul JY, Song IS and Kim JY, **2011**. Chrysophanic acid blocks the proliferation of colon cancer cells by inhibiting EGFR/mTOR pathways. *Phytotherapy Research*. **25**:833-837.
- Lim W, An Y, Yang C, Bazer FW and Song G, **2018**. Chrysophanol induces cell death and inhibits invasiveness via mitochondrial calcium overload in ovarian cancer cells. *Journal of Cellular Biochemistry*. **2018**:1-12.
- Lin L, Huang L, Lin C, Lee C, Chen JL, Hsu MS and Lin S, **2005**. Determination of Binding Constant of DNA-binding Drug to Target DNA by Surface Plasmon Biosensor Technology. *Current Drug Targets-Immune, Endocrine & Metabolic Disorders*. **5**:61-72.
- Li N, Ma Y, Guo L and Yang X, **2005**. Interaction of anticancer drug mitoxantrone with DNA analyzed by electrochemical and spectroscopic methods, *Biophysical Chemistry*. **116**:199–205.
- Luedtke NW, **2009**. Targeting G-quadruplex DNA with Small molecules. *Chimia*. **63**:134-139.

- Malgowska M, Gudanis D, Teubart A, Dominiak G and Gdaniec Z, **2012**. How to study G-quadruplex structures. *Journal of Biotechnology, Computational Biology and Bionanotechnology*. **93:**381-390.
- Malhotra BD and Chaube A, 2003. Biosensors for clinical diagnostics industry. *Sensors Actuators B Chemical*. **91**:117–126.
- Malik EM and Muller EC, **2016**. Anthraquinones as pharmacological tools and drugs. *Medicinal Research Reviews*. **4**:705-748.
- Mender I and Shay JW, 2015. Telomerase Repeat Amplification protocol (TRAP). *Bio-protocol*.
- Nagesh N, Sharma V, Kumar G and Lewis E, **2009**. Effect of Porphyrin Drugs Interaction with Quadruplex DNA Formed by the Promoter Region of C-myc and Bcl2 Oncogenes. *Journal of Nucleic Acids*. **2010**:1-9.
- Nam JM, Thaxton CS and Mirkin CA 2003. Nanoparticle based bio-bar codes for the ultrasensitive detection of proteins. *Science*. **301**:1884-1886.
- Owen PM and William IR, 2010. Shape and surface area measurements using scanning electron microscope stereo-pair images of volcanic ash particles. *Geosphere*. **6**:1-7.
- Parkinson G, Lee MPH and Neidle S, **2002**. Crystal Structure of Parallel Quadruplexes From Human Telomeric DNA. *Nature*. **417**:876-880.
- Perry P, Gowan S, Reszka A, Polucci P, Jenkins T, Kelland L and Neidle S, **1998**. 1,4- and 2,6-Disubstituted Amidoanthracene-9,10-dione derivatives as Inhibitors of Human Telomerase. *Journal of Medicinal Chemistry*. **41**:3253-3260.
- Phan A, **2009**. Human Telomeric G-Quadruplex: Structures of DNA and RNA sequences. *Federation of European Biochemical Societies*. **277**:1107-1117.
- Pradeep TP and Barthwal R, **2016**. NMR structure of dual site binding of mitoxantrone dimer to opposite grooves of parallel stranded G- quadruplex [d-$(TTGGGGT)_4$]. *Biochimie*. 59-69.

- Qiao C, Bi S, Sun Y, Song D, Zhang H and Zhou W, **2007**. Study of Interactions of Anthraquinones with DNA using Ethidium Bromide as a Fluorescence Probe. *Spectrochimica Acta.* **70**:136-143.
- Qiao Y, Deng J, Jin Y, Chen G and Wang L, **2012**. Identifying G-quadruplex-binding ligands using DNA-functionalized gold nanoparticles. *The Royal Society of Chemistry*. **137**:1663-1668.
- Qin Y and Hurley LH, **2008**. Structures, folding patterns and functions of intramolecular DNA G-quadruplexes found in eukaryotic promoter regions. *Biochimie*. **90**:1149-1171.
- Raje S and Barthwal R, **2019**. Molecular recognition of 3+1 hybrid human telomeric G-quadruplex DNA d-[AGGG(TTAGGG)$_3$] by anti-cancer drugs epirubicin and adriamycin leads to thermal stabilization. *International Journal of Biological Macromolecules*.**139**:1272-1287.
- Rhodes D and Lipps H, **2015**. G-Quadruplexes and their Regulatory Roles in Biology. *Nucleic Acids Research.* **43**:8627-8637.
- Ruiz GV, Olives AI, Martin MA, Ribelles P, Ramos MT and Menendez JC, **2011**. An overview of analytical techniques employed to evidence Drug–DNA interactions. Applications to the design of Genosensors. *Biomedical Engineering, Trends, Research and Technologies*. **3**:65–90.
- Sirajuddin M, Ali S and Badshah A, **2013**. Drug-DNA interactions and their Study by UV-Visible, Fluorescence Spectroscopies and Cyclic Voltametry. *Journal of Photochemistry an*d *Photobiology B:Biology*. **124**:1-19.
- Tariq Z and Barthwal R, **2018**. Binding of Anticancer Drug Daunomycin to Parallel G-Quadruplex DNA [d-(TTGGGGT)4] leads to Thermal Stabilization: A Multispectroscopic Investigation. *International Journal of Biological Macromolecules*. **120**:1965-1974.
- Tripathi S, Pradeep PT and Barthwal R, **2016**. Molecular recognition of Parallel DNA Quadruplex d-(TTAGGGT) by Mitoxantrone: Binding with 1:2 Stoichiometry leading to Thermal Stabilization and Telomerase Inhibition. *Chem Bio Chem*. **17**:554-560.

- Tyagi H, Kushwaha A, Kumar A and Aslam M, **2016**. A Facile pH-controlled Citrate-Based Reduction Method for Gold Nanoparticles Synthesis at Room Temperature. *Nanoscale Research Letters.* **11**:362-373.
- Unser S, Bruzas I, He J and Sagle L, **2015**. Localised Surface Plasmon Resonance Biosensing: Current Challenges and Approaches. *Sensors*. **15**:15684-15716.
- Wang Y and Patel DJ, **1993**. Solution Structure of Human Telomeric Repeat d-[$AG_3(T_2AG_3)_3$] G-Tetraplex. *National Center for Biotechnology Information.* **1**:263-82.
- Xu Y, **2011**. Chemistry in Human Telomere Biology: Structure, Function, and Targeting of Telomere DNA/RNA. *Chemical Society Reviews.* **40**:2719-2740.
- Yang D and Okamoto K, **2010**. Structural Insights into G-Quadruplexes: Towards new Anticancer Drugs. *Future Medicinal Chemistry*. **2**:619-646.
- Ye M, Yao G, Wei J, Pan Y, Liao Z and Wang H, **2013**. Synthesis, Cytotoxicity, DNA binding and apoptosis of Rhein-Phosphonate derivatives as antitumor agents. *International Journal of Molecular Sciences*. **14**:9424-9439.